GENETIC MARKERS WHICH IDENTIFY INDIVIDUALS WHO IMPROVE THEIR CHOLESTEROL LEVELS AND DIABETES STATUS WITH EXERCISE

FIELD OF THE INVENTION

The present invention relates to identifying one or more genetic markers which correlate with greater success in improving cholesterol levels and diabetes status in individuals with and without high cholesterol levels or diabetes.

BACKGROUND OF THE INVENTION

Studies have shown that individuals suffering from or at risk of developing high cholesterol levels or diabetes can alleviate symptoms or otherwise improve their conditions through exercise. Unfortunately, some individuals, no matter how rigorously they exercise, are unable to improve their conditions, while others benefit to a much greater extent than predicted. These results underscore the fact that many factors contribute to an individual's well-being. Such factors include, for example, behaviors such as diet and exercise, genetic makeup, and environment. While behavior and environment can be controlled, altered or regulated, an individual's genetic makeup is essentially predetermined and set at birth. The present inventors hypothesized that upon identifying the genetic makeup of a population suffering from or at risk of developing high cholesterol levels or diabetes and observing that some individuals of the population improve their cholesterol levels and diabetic status from a change of behavior to a much greater or lesser extent than expected, a correlation could be made between the presence or absence of certain genetic markers and success in improving cholesterol levels and diabetic status.

An object of the present invention is to identify one or more genetic markers which positively correlate with greater success in improving cholesterol levels and diabetes status in individuals with and without high cholesterol levels or diabetes.

SUMMARY OF THE INVENTION

The present inventors have discovered a number of genetic markers which positively correlate with greater success in improving cholesterol levels and diabetes status in diabetic, hypercholesteremic or at risk individuals, as compared with other genetic makeup at the same gene loci. Therefore, a first embodiment of the present invention is directed to a method of improving cholesterol levels in a subject with increased cholesterol levels or at risk of developing such a condition, the method comprising:

identifying a subject with hypercholesteremia or at risk of developing such a condition having an allele and/or a genotype at a gene locus which positively correlates with greater success in improving cholesterol levels in hypercholesteremic individuals, as compared with other alleles and/or genotypes at the same gene locus; and

engaging the subject in exercise training for a period of time sufficient to improve the cholesterol levels in the subject.

A second embodiment of the present invention is directed to a method of improving diabetes status in a subject with diabetes or at risk of developing diabetes, the method comprising:

identifying a subject with diabetes or at risk of developing diabetes having an allele and/or a genotype at a gene locus which positively correlates with greater success in improving diabetes status in diabetic individuals, as compared with other alleles and/or genotypes at the same gene locus; and

engaging the subject in exercise training for a period of time sufficient to improve the diabetes status in the subject.

DETAILED DESCRIPTION OF THE INVENTION

The inventors have found that a number of genetic markers positively correlate with greater success in improving cholesterol levels or diabetes status in individuals with hypercholesteremia or diabetes, or at risk of developing such disorders, as compared with other genetic makeup at the same gene loci.

Markers which the inventors have investigated include the glucose transport 4 (GLUT4) gene, the myostatin gene and the insulin receptor substrate-1 (IRS-1) gene.

The term "improved cholesterol levels" means an improvement in at least one characteristic area which is associated with hypercholesteremia. An improvement may be in one or more of the following characteristic areas (this list is non-exhaustive and includes overlapping and representative examples only): change in cholesterol metabolism, increase in high density lipoprotein cholesterol (HDL-C) levels, increase in high density lipoprotein cholesterol 2 (HDL_2-C) levels, decrease in low density lipoprotein cholesterol (LDL-C) levels or increase in the ratio of HDL-C or HDL_2-C levels as compared to LDL-C levels. These improvements may be measured by, for example, plasma cholesterol tests conducted before and after exercise training. An improvement in cholesterol levels in accordance with the invention may be found both in individuals with hypercholesteremia and in individuals at risk of developing such a disorder.

The term "improved diabetes status" means an improvement in at least one characteristic area which is associated with diabetes. An improvement may be in one or more of the following characteristic areas (this list is non-exhaustive and includes overlapping and representative examples only): change in glucose metabolism, change in insulin metabolism, change in glucose levels from a baseline determination, change in insulin levels from a baseline determination, change in fasting plasma glucose levels, change in fasting plasma insulin levels or change in acute insulin response to glucose. These improvements may be measured by, for example, glucose tolerance tests conducted before and after exercise training. An improvement in diabetes status in accordance with the invention may be found both in individuals with diabetes and in individuals at risk of developing diabetes.

The term "single course of exercise", as used throughout this application, means a cardiovascular exercise session of any type which is conducted during

one day. An exercise session may comprise an aerobics class, treadmill training, step machine, or any other suitable cardiovascular exercise regimen. For most cases, exercise may be completed in, for example, 30 minutes to 3 hours, with optional brief rest periods of 3-15 minutes, however this amount would vary depending on the health and endurance of the subject.

The term "extensive exercise" means about 10 single courses of exercise or more, preferably at least 15, at least 20, or at least 25 single courses of exercise, over a defined period of time ("the exercise period"). The exercise period in the case of an extensive exercise protocol may be from about 50-400 days, preferably from about 70-350 days or 100-300 days.

The time between exercise courses depends on the health and endurance of the subject. Preferably, the time between exercise courses may be from about 1-3 days or more.

The present inventors have discovered that hypercholesteremic or diabetic individuals or those at risk of developing hypercholesteremia or diabetes with different genotypes for genes which control the manufacture of certain proteins exhibit different degrees of success in improving their cholesterol levels and diabetes status through exercise. The inventors have surprisingly discovered that each genotype potentially can benefit from exercise, however, the amount of exercise which produces the most benefits varies according to genotype. These results could not have been predicted from initial patient screening.

Glucose transport in skeletal muscle is mainly facilitated by the insulin-responsive GLUT4. In the basal state, GLUT4 is stored in a transporter-enriched intracellular pool. Following insulin stimulation, GLUT4 is translocated from the intracellular compartment to both the plasma membrane and the T-tubules.

The inventors have found that subjects having a BamHI "AA" genotype for a GLUT4 gene exhibit a greater improvement in cholesterol levels than those with a "GG" or "AG" genotype, after extensive exercise.

Therefore, one method of improving cholesterol levels in a subject in need of such improvement according to the invention comprises identifying a subject having a BamHI "AA" genotype for a GLUT4 gene, wherein the subject is in need of improved cholesterol levels and engaging the subject in extensive exercise training for a period of time sufficient to improve the cholesterol levels in the subject.

Myostatin, also known as growth/differentiation factor-8 (Gdf8), is a member of the transforming growth factor-beta (TGF-β) superfamily, which encompasses a large number of growth and differentiation factors that play important roles in regulating embryonic development and in maintaining tissue homeostasis in adult animals. During early stages of embryogenesis, myostatin expression is restricted to the myotome compartment of developing somites. At later stages and in adult animals, myostatin is expressed in many different muscles throughout the body.

The inventors have found that subjects having a "12" genotype for exon 2 of the myostatin gene exhibit a greater improvement in cholesterol levels than those with a "11" genotype, after extensive exercise.

Therefore, another method of improving cholesterol levels in a subject in need of such improvement according to the invention comprises identifying a subject having a "12" genotype for exon 2 of a myostatin gene, wherein the subject is in need of improved cholesterol levels and engaging the subject in extensive exercise training for a period of time sufficient to improve the cholesterol levels in the subject.

The inventors have also found that diabetics or those at risk of developing diabetes having a "11" genotype at exon 2 of the myostatin gene improve their diabetes status more with extensive exercise training than those having a "12" genotype.

Therefore, a method of improving diabetes status in a subject in need of such improvement comprises identifying a subject having a "11" genotype for exon 2 of a myostatin gene, wherein the subject is in need of improved diabetes

status and engaging the subject in extensive exercise training for a period of time sufficient to improve the diabetes status in the subject.

IRS-1 is a 185 kDa protein which is activated rapidly upon insulin stimulation of cells, and is a key mediator of an insulin-regulated biological activity. The amino-terminal region of the protein contains interaction modules that facilitate its binding to receptors of insulin. The remainder of the molecule contains numerous tyrosine containing motifs, which, when phosphorylated by the insulin receptor tyrosine kinase, serve as binding regions for a variety of cellular proteins containing a so-called "SH2" domain.

The inventors have found that subjects having a "12" genotype for the IRS-1 gene exhibit a greater improvement in cholesterol levels than those with a "11" genotype, after extensive exercise.

Therefore, in accordance with this aspect of the present invention, a method of improving cholesterol levels in a subject in need of such improvement comprises identifying a subject having a "12" genotype for an IRS-1 gene, wherein the subject is in need of improved cholesterol levels and engaging the subject in extensive exercise training for a period of time sufficient to improve the cholesterol levels in the subject.

EXAMPLES

Example 1. Variations in Improvement of Cholesterol Levels in Subjects with Different GLUT4 BamHI, Myostatin and IRS-1 Genotypes After Extensive Exercise

DNA was obtained from obese sedentary men 50-65 yrs of age, and processed as follows.

Detection of (C581T) and (A30G) Substitution in GLUT4

Genotyping for the (C581T) and (A30G) substitutions in GLUT4 was performed by amplification using sense primer 5'-CAGTGCCCGGAGCAGGGAGGCGCT-3' and antisense primer 5'-

GCGAAGATGAAAGAACCGATCCTG-3' followed by digestion with the restriction endonucleases AvaII and BamHI, respectively. The presence of a C at np-518 yields major AvaII restriction fragments of 408 and 378 base pairs and the presence of a T at np-518 yields fragments of 119, 289 and 378 base pairs. The presence of an A at np-30 yields major BamHI fragments of 389 and 342 base pairs, while the presence of a G at np-30 yields fragments of 445 and 389 base pairs. All denotations of sequence positions are based on those recited in Bjorbaek et al. (1994), *Diabetes*, 43:976-983, hereby incorporated by reference.

Detection of Lys153Arg Substitution in Myostatin Exon 2

DNA amplification primers for exon 2 of the human myostatin gene were designed based on the cDNA sequence of human myostatin (GenBank Accession No. AF019627) and the genomic organization of the bovine myostatin gene (Grobert et al. (1998), *Mamm. Gen.* 9:210-213, incorportated by reference). Amplimers were sequenced directly using the dRhodamine ready reaction kit (Perkin Elmer) and analyzed on the ABI Prism Model 377 (Applied Biosystems) fluorescent sequencer. Sequences were aligned for comparison using SEQUENCHER™ 3.0 (Gene Codes). Primer 1 had the sequence 5'-GAAAACCCAAATGTTGCTTC-3', and primer 2 had the sequence 5'-TGTCTAGCTTATGAGCTTAGGG-3'. The temperature was 54°C, and the buffer was 2.0 mM MgCl. PCR products were digested with BanII and the digested products were run on 2% agarose gels.

Detection of Gly972Arg Substitution in IRS-1

A 220 bp region encompassing the Gly972Arg substitution was amplified from approximately 20 ng of genomic DNA with upstream primer 5'-GCAGCCTGGCAGGAGAGCCAT-3' and downstream primer 5'-CTCACCTCCTCTGCAGCAATG-3'. PCR products were digested with BstNI. The digested products were run on a 4% agarose gel, stained with

ethidium bromide, and visualized by UV transillumination. The expected digestion product sizes were 220 bp for Gly972 homozygotes, 164 bp and 56 bp for Arg972 homozygotes and 220 bp, 164 bp and 56 bp for heterozygotes.

Results

The subjects underwent 9 months of endurance exercise training to quantify, among other things, their improvements in plasma cholesterol levels. Subjects were initially stabilized on an American Heart Association low-fat diet and had fasting blood samples drawn for plasma cholesterol measurements. This diet was maintained throughout the 9 months of exercise training and subjects repeated the blood sampling for cholesterol levels after training. The data in Table 1 represent the change in HDL-C and HDL_2-C levels that occurred with exercise training. Subjects with the GLUT4 BamHI "AA" genotype increased their plasma HDL-C and HDL_2-C levels with exercise training substantially more than subjects with the GLUT4 BamHI "GG" or "AG" genotype. Furthermore, subjects with the myostatin exon 2 "12" genotype increased their plasma HDL-C and HDL_2-C levels with exercise training substantially more than subjects with the myostatin exon 2 "11" genotype. Lastly, subjects with the IRS-1 "12" genotype increased their plasma HDL-C and HDL_2-C levels with exercise training substantially more than subjects with the IRS-1 "11" genotype. Thus, these results indicate that GLUT4, myostatin exon 2 and IRS-1 genotypes identify those individuals most likely to improve their cholesterol levels with exercise training.

Table 1: Change with Exercise Training in Plasma Lipoprotein Levels as a Function of Genotype

	Change with Exercise Training	
GLUT4 BamHI	**HDL-C**	**HDL_2-C**

GG and AG genotype (n=13)	1.9 ± 3.8	0.7 ± 4.0
AA genotype (n=2)	16.9 ± 12.9	11.1 ± 15.0
Myostatin Exon 2		
11 genotype (n=13)	2.0 ± 4.0	0.4 ± 4.0
12 genotype (n=2)	15.8 ± 14.4	13.6 ± 11.5
IRS-1		
11 genotype (n=10)	2.9 ± 1.4	-0.1 ± 1.5
12 genotype (n=3)	11.4 ± 7.3	8.5 ± 6.6

Values are mean ± SD. Values are expressed as the change with 9 months of exercise training in HDL-C and HDL_2-C levels. Thus, positive values indicate a response that is greater after training.

Example 2. Variations in Improvement of Diabetes Status in Subjects with Different Myostatin Exon 2 Genotypes After Extensive Exercise

The subjects, detection of polymorphisms and the exercise regimen for these subjects was described in Example 1. Subjects underwent an oral glucose tolerance test with blood samples drawn for up to 3 hours after the ingestion of a standard glucose load. Subjects repeated the glucose tolerance test after training. The data in the following Table 2 represent the change in the integrated glucose area above baseline that occurred with the exercise training. Subjects with the myostatin exon 2 "11" genotype decreased their glucose areas more with exercise training than subjects with the myostatin exon 2 "12" genotype. These results indicate that myostatin exon 2 genotypes identify those individuals most likely to improve their diabetes status with exercise training.

Table 2: Change with Exercise Training in Integrated Glucose Area in Response to an Oral Glucose Tolerance Test as a Function of Genotype

Myostatin Exon 2 Genotype	Change with Exercise Training in Glucose Area
11 genotype (n=14)	-1941 ± 1260
12 genotype (n=3)	1180 ± 1641

Values are mean ± SD. Values are expressed as the change with 9 months of exercise training in integrated glucose area above baseline for 3 hours following a standard oral glucose challenge. Negative values indicate a response that is reduced after exercise training and positive values a response that is greater after training.